A Study of the Respiration and of the Function of Hæmoglobin in *Planorbis corneus* and *Arenicola marina*.

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With 15 Figures in the Text.

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INTRODUCTION.

THE presence of hæmoglobin in the invertebrates has generally been found to be correlated with a habitat at times deficient in oxygen; the function assigned to the hæmoglobin being either that of storing oxygen, or of transporting it at low pressures. Thus Leitch (1916) has shown that in Planorbis the hæmoglobin is used only when the oxygen pressure is so low that the necessary amount cannot be supplied by diffusion. Barcroft and Barcroft (1924) suggested that in Arenicola it acts "as a reserve store which the organism can use up when it has not access to sea-water." This

indicates that in Planorbis and Arenicola, both of which have hæmoglobin dissolved in the plasma and are adapted to environments in which the oxygen concentration must at times be considerably reduced, the function of the hæmoglobin is different.

Hill (1913–24), Meyerhof (1920) and others have shown that the fundamental mechanism of muscular contraction is an anaerobic one, by which nearly the whole of the energy is liberated in the breakdown of carbohydrate, as glycogen, to lactic acid ; while the recovery process, consisting of the restoration of a portion of the lactic acid to glycogen, is aerobic. It has also been proved that when an isolated muscle is stimulated in an oxygen-free atmosphere lactic acid accumulates, and the tissue goes into debt for oxygen, the magnitude of the debt being proportional to the quantity of the acid formed.

Slater and Davis (1926–28) have demonstrated that this phenomenon occurs also in the whole animal. They found that the common cockroach and the earthworm, when subjected to oxygen lack, went into debt and by this means were able to survive short periods of anaerobiosis.

It appears, therefore, that there are two mechanisms by which an animal can obtain the oxygen necessary for its life. Firstly, the circulatory system functioning under normal conditions ; secondly, the initial phase of muscular metabolism providing an emergency mechanism by which the animal is tided over short critical periods of oxygen deficiency.

In view of these facts it seemed of interest to study the respiration of, and the function of hæmoglobin in, Planorbis and Arenicola, and to find more exactly the significance of the hæmoglobin. Is it for the latter a storer of oxygen and for the former a transporter at low pressures, or are the animals able to go into oxygen debt and, therefore, not dependent on their blood for a supply of oxygen during anaerobiosis ?

EXPERIMENTAL METHODS AND RESULTS.

A. *PLANORBIS CORNEUS.*

1. Normal Rate of Respiration.

The respiratory rate was measured by observing the oxygen intake in Barcroft's differential blood gas apparatus (1914). Small snails weighing about 0·5 gm. were used, one animal being used for each experiment. The snail, having been first lightly dried to remove moisture and organic matter from the shell, was weighed and placed in the right-hand flask of the apparatus. About 2 c.c. of water were added to each flask, a slight additional amount being put in the left-hand flask to compensate for the volume of the snail. In the reservoir of each was placed a small piece of caustic soda to absorb carbon dioxide. The flasks were then attached to the U-shaped manometer and the apparatus set shaking in a water-bath,

the temperature of which closely approximated that of the room and was constant to within 0·5° of 15° C. The oxygen intake was recorded every 15 minutes for several hours, ample time having been first allowed for conditions of equilibrium to be established within the apparatus.

The average oxygen intake for a number of animals was 0·026 c.c. per gm. per hour. The results from which this figure was obtained are expressed in Table I.

TABLE I.

No. of Animal.	Weight gm.	Oxygen consumption in c.c. per hour. 1	2	3	4	5	6	Average in c.c. per gm. per hr.
1.	0·49	0·022	0·015	0·021	0·017	0·024	0·019	0·040
2.	0·62	0·016	0·014	0·014	0·012	0·018	—	0·024
	0·65	0·017	0·021	0·014	0·017	0·013	0·016	0·025
	0·66	0·017	0·017	0·010	0·018	0·015	0·017	0·024
3.	0·68	0·020	0·015	0·020	0·022	0·020	0·020	0·029
	0·69	0·028	0·024	0·027	0·027	0·020	0·022	0·036
	0·71	0·013	0·011	0·016	0·012	0·020	0·014	0·020
4.	0·68	0·017	0·013	0·014	0·015	0·013	0·012	0·021
5.	0·70	0·012	0·011	0·013	0·013	—	—	0·018

It may be seen from the above Table that the oxygen consumption of a snail weighing 0·49 gm. was 0·040 c.c. per gm. per hour, while that of snails weighing on the average 0·67 gm. was 0·024 c.c. per gm. per hour, or 0·016 c.c. less than that of the smaller animal. It is thought that, as in the case of the cockroach (Davis and Slater, 1926), the apparent relationship between the body weight and oxygen consumption is in reality a correlation between age and oxygen intake, the younger animals having a quicker metabolic rate. The oxygen consumption of two snails, Nos. 2 and 3 respectively, was measured for each on three different occasions. The results for each are fairly uniform.

Figure 1 represents the oxygen intake measured at 15° C. over a period of six hours, for a snail weighing 0·64 gm.

2. The Blood Volume.

Estimations of the blood volume were made in a Duboscq colorimeter. The snail was bled as thoroughly as possible, the blood being collected from the pulmonary cavity into which it had been driven by an artificially stimulated contraction of the foot. About 0·5 c.c. was thus obtained.

A known volume of blood was diluted to 10 c.c. with distilled water and used as the standard solution in the colorimeter. The rest of the snail was chopped up under a known volume of water to extract the blood from its tissues, and the wash-water was then filtered from the macerated

tissue by suction through a Buchner filter. Ten cubic centimetres of the diluted blood were then matched against the standard solution in the colorimeter, and the concentration of hæmoglobin in the sample of wash-water estimated according to the formula $C_1 = \frac{R_2}{R_1} \times C_2$, where R_1 and R_2 are the readings on the colorimeter of the unknown and standard solutions; C_1 and C_2 their respective concentrations. Care was taken always

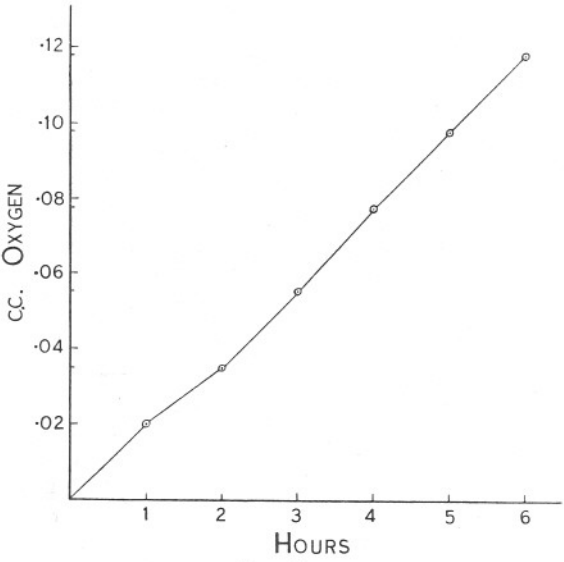

Fig. 1.

to use the same calibrated pipettes for measuring the solutions. The data of one estimation are tabulated as follows :—

TABLE II.

Showing Estimation of Total Blood in One Snail.

Weight of snail 1·61 gm.
Undiluted blood 0·42 c.c. made up to 4·2% aqueous solution.
Wash-water 18·65 c.c.

Colorimetric Readings.

No.	R_2	R_1	C_2	C_1
1.	1·27	1·65	4·2	3·23
2.	1·13	1·48	4·2	3·21
3.	1·08	1·37	4·2	3·31
4.	1·22	1·60	4·2	3·20
5.	1·23	1·54	4·2	3·35

Mean Value for C_1, 3·26%, therefore in 18·65 c.c. wash-water there is 0·61 c.c. blood, and the total volume of blood in the snail is 1·03 c.c.

The results of the experiments in Table III show the average blood volume to be 0·58 c.c. per gm.

TABLE III.

No.	Weight of Snail in grams.	Volume of Blood in c.c.	Volume of Blood per gm. of Snail.
1.	0·75	0·47	0·63
2.	1·10	0·74	0·67
3.	1·14	0·86	0·75
4.	1·44	1·00	0·69
5.	1·54	0·79	0·40
6.	1·56	0·98	0·63
7.	1·58	0·68	0·43
8.	1·61	1·03	0·65
9.	1·83	0·83	0·45
10.	1·90	0·98	0·51

3. The Oxygen Capacity of the Blood.

The oxygen capacity of the hæmoglobin was estimated by the ferricyanide method in Barcroft's apparatus and at a later date additional experiments were made in Van Slyke's manometric gas apparatus.

The experimental procedure was similar to that employed by Barcroft (1914), and Van Slyke and Neill (1924) respectively. A slight modification of the Barcroft apparatus was, however, introduced by using a small glass tube, as suggested by Keilin (1928, p. 217), to hold the ferricyanide solution in place of the reservoir in each flask more generally used for that purpose. The tube was hooked on to the reservoir in such a manner that the contents, and if desirable the tube itself, could be upset into the flask. This proved a more convenient method of bringing the ferricyanide in contact with the blood as, owing to the small amount of reagent used, great difficulty was formerly experienced in overcoming the effect of surface tension in the narrow reservoir.

The snails were bled as described above, undiluted blood from several individuals being mixed and well shaken to saturate the blood with oxygen. One cubic centimetre of the mixture was used for each measurement of the oxygen capacity.

The experiments showed that in 1 c.c. of blood the amount of oxygen held by the hæmoglobin was approximately 0·013 c.c., the total oxygen content of the blood 0·014 c.c. The results from which these values were obtained are given in Table IV.

TABLE IV.

ESTIMATIONS MADE ON 1 C.C. OF BLOOD.

	Combined Oxygen by Barcroft's Apparatus in c.c.	Total Oxygen Capacity of Blood by Van Slyke's Apparatus in c.c.
	0·011	0·015
	0·010	0·014
	0·013	0·014
	0·011	—
	0·015	—
	0·016	—
Mean	0·013	0·014

The length of time that the total oxygen content of the blood will last the animal was calculated as follows :—

Blood volume per gm. of snail	0·581 c.c.
Oxygen content per c.c. blood	0·014 c.c.
Oxygen capacity of blood per gm. of snail	0·0081 c.c.
Oxygen consumption per gm. per hour	0·026 c.c.

Hence oxygen content of blood will last the animal 18 minutes.

4. SPECTROSCOPIC EXAMINATION OF THE HÆMOGLOBIN.

(*a*) *Under Anaerobiosis*

Although the oxygen reserve of the blood and the extent to which it serves as a store of oxygen had been estimated, it seemed desirable to make a direct observation of the blood pigment during anaerobiosis. Stated briefly, the method of obtaining the information consisted of placing a number of animals in an inert gas ; obtaining and examining a sample of blood at frequent intervals ; noting at what time the bands of oxyhæmoglobin were replaced by that of reduced hæmoglobin. The examination of the blood was in every instance made under anaerobic conditions.

The apparatus for examining the blood was comprised of a micro-spectroscope fitted to a compound microscope. This, with the animals, was housed in a large box into which a steady flow of nitrogen could be maintained. Figure 2 illustrates the general structure of the box, the more particular features of which, however, require a brief description.

The back and upper half of the front were of glass, the rest of the box being made of wood, one inch in thickness. The top was removable to allow for the entrance of the microscope which, when once in place, was not withdrawn until the conclusion of the investigation. The top could be screwed down firmly and the junction between it and the rest of the box

was made air-tight by means of putty. To allow for focussing the microscope, the draw-tube projected through an opening in the top, the opening being lined with baize, so that the tube fitted snugly and yet could be moved up or down. At the bottom right-hand side was situated an inlet tube for nitrogen, the outlet being located on the opposite side near the top. Near the inlet tube a small door was cut, and by means of this opening the animals were admitted to and withdrawn from the box. The aperture into which the door fitted was lined with baize, and during the experiments when the door was shut the outside was covered with adhesive tape to prevent the leakage of nitrogen through the cracks, the pressure of nitrogen inside the box tending to be greater than atmospheric. In the lower half of the front two fairly large holes were made and over each the wrist part of a rubber glove was stretched and held in place by being firmly nailed, between two strips of elastic, to the wood. Seccotine was then run round the outside edge of the join which proved to be air-tight. By means of the gloves it was possible, without letting in any air, to put one's hands inside the box and thus manipulate the animals. Both the inner and the outer surfaces of the box were painted, the inside also being coated with paraffin wax.*

The nitrogen used for producing an oxygen-free atmosphere was filtered on its way to the box through alkaline hydrosulphite and alkaline solutions of pyrogallol to remove the traces of oxygen usually present in the commercially prepared gas. At the commencement of each experiment the air inside the box was swept out with nitrogen for half an hour or longer, the outlet tube was then closed, the door opened and the animals quickly placed inside. A constant flow of nitrogen was maintained throughout the experiment, the excess escaping around the draw-tube of the microscope. The atmosphere in the box was frequently tested for oxygen by Haldane's gas analysis apparatus. A connection between the latter and the outlet tube had been established with pressure tubing. Tests showed that the atmosphere in the box was never entirely anaerobic, the partial pressure of oxygen being approximately 0·5 mm.

At frequent intervals separate snails were bled directly on to a clean glass slide, and the blood examined for the presence of reduced hæmoglobin. The whole operation of bleeding the snail and noting the condition of the hæmoglobin took only a few seconds.

On account of the ability of Planorbis to hold a quantity of air in its lung it was necessary, before placing the snails in the nitrogen-box, to expose them for a few minutes to a vacuum. The investigation of the time taken for the blood to be reduced was also performed on snails in

* I am indebted to the Department of Botany, University College, London, for the use of the microspectroscope and to Mr. A. G. Nicholls for the construction of the nitrogen-box.

which the air had not been removed from the lung. Comparison of the results of these two types of experiments revealed the extent to which the lung supplemented the blood as a store for oxygen.

The results are given in Figure 3.

A survey of the investigation shows that for the experiments in which the snails were without air in their lungs the hæmoglobin was fully reduced, in some instances after subjection to only 10 minutes anaerobiosis, and in all cases some reduction had occurred; while after 25 minutes it was,

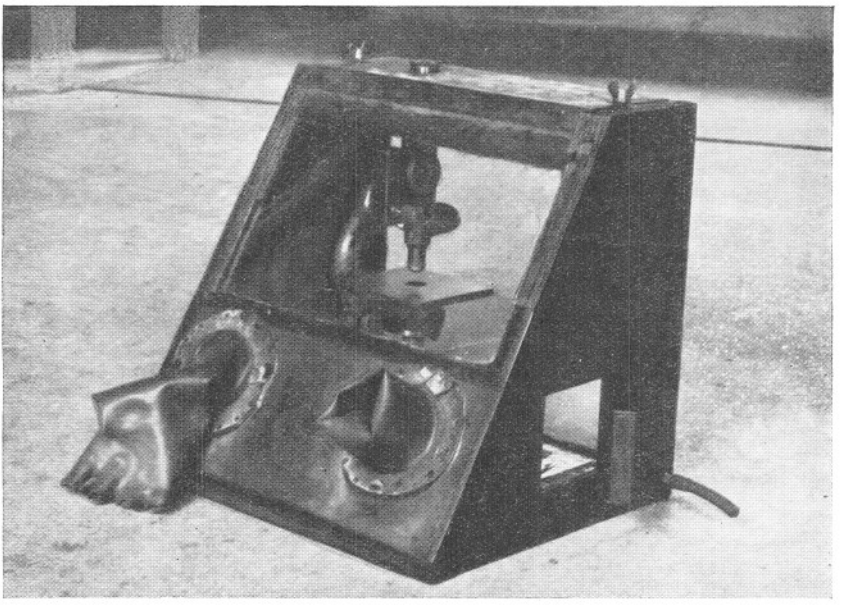

FIG. 2.

with two exceptions, fully reduced. If, however, the snails were used with their lungs full of air, the blood remained in the form of oxyhæmoglobin for 40 minutes, while between 40 and 80 minutes both the oxy- and reduced forms of hæmoglobin were found. After 80 minutes it was, with a few exceptions, completely reduced.

It is, therefore, clear that the total oxygen supply in the blood will last from approximately 20 to 25 minutes, and this is in fairly close agreement with the theoretically determined value of 18 minutes. The air in the lung will supply oxygen for 40 minutes, so that altogether Planorbis is well able to endure an hour's subjection to oxygen deficiency.

The experiments also substantiate Leitch's evidence that the combined oxygen is the last to be used by the animal but, whereas she found only

enough for three minutes and did not think it constituted a reserve, this investigation indicates that it may indeed be a reserve of some importance, since the oxygen supplied by the blood considerably prolonged the animal's survival in an anaerobic atmosphere.

(b) Under Aerobiosis following Anaerobiosis.

It was important with reference to subsequent oxygen debt experiments to ascertain if the animals, having been exposed to an anaerobic atmosphere, oxidised their hæmoglobin immediately on admittance to air. If

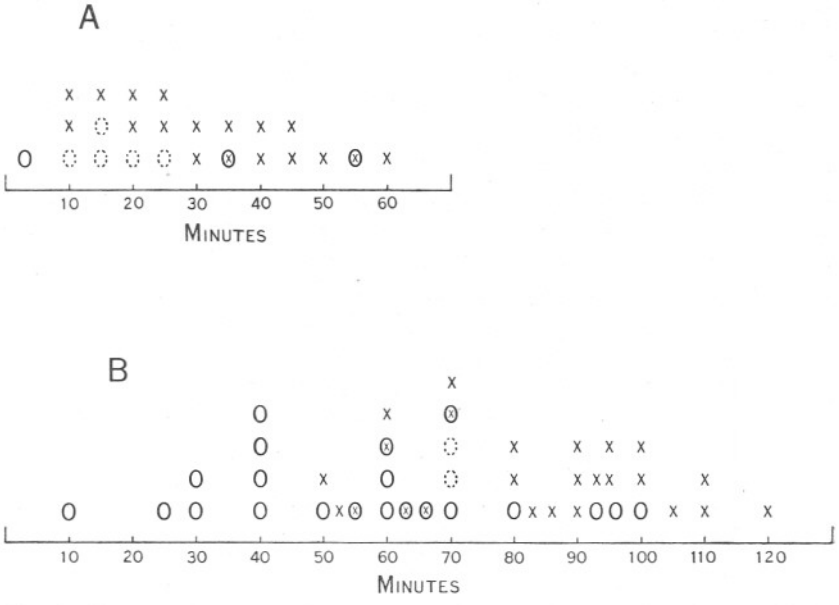

FIG. 3.—Diagram illustrating the time taken for the reduction of oxyhæmoglobin in Planorbis; A, in snails in which air had been removed from lungs; B, in snails with air in lungs. Separate animals were examined at frequent intervals. O, indicates presence of oxyhæmoglobin; ◌, presence of partially reduced hæmoglobin; ⊗, presence of almost completely reduced hæmoglobin; X, presence of reduced hæmoglobin.

N.B. The condition designated as partially reduced hæmoglobin was that in which the bands of oxyhæmoglobin were very faint; that of almost completely reduced hæmoglobin in which the faint oxyhæmoglobin bands were almost entirely masked by a weak reduced hæmoglobin band.

oxidation were not almost instantaneous the amount thus used would be included in the oxygen intake, thus giving a false value to the oxygen debt.

These experiments were, therefore, undertaken to measure the time taken by the animals for oxidising their hæmoglobin. For this purpose it was again necessary to carry out in the nitrogen-box the procedure of

exposing the animals to oxygen deficiency until the blood was reduced. They were then admitted to air in groups of two for varying periods, the blood being, however, collected and examined under anaerobic conditions. An additional piece of apparatus, a small stoppered bottle, placed on the floor of the nitrogen-box, was so arranged by means of an inlet tube that it could be connected directly with either the nitrogen supply or compressed air, while an outlet tube, extending to the exterior of the box, provided an escape for whatever gas was run into the bottle.

The experimental technique was as follows : nitrogen having been run into the box and into the bottle sufficiently to expel the air, the outlet tubes were closed and the animals placed in the box, the flow of nitrogen being continuous.

The blood from several snails was inspected until the hæmoglobin was found to be reduced ; two were then exposed to a current of air in the bottle for a definite time, at the end of which they were removed and the blood examined. Before removing the snails, however, the air in the bottle was expelled by a flow of nitrogen so that there was no introduction of air into the nitrogen-box. The details of a typical experiment are given in Table V, and the collective results in Figure 4.

TABLE V.

R indicates reduced hæmoglobin ; O, oxyhæmoglobin.

No.	Period of Anaerobiosis in min.	Time in Air in min.	Condition of Hæmoglobin.
1.	60	0	R
2.	62	0	R
3.	65	2	R
4.	65	2	R
5.	90	5	R
6.	90	5	R
7.	105	10	R
8.	105	10	R
9.	135	30	O
10.	135	30	O

During the above experiment the partial pressure of oxygen in the box was estimated after 45, 75, and 120 minutes, the values obtained being respectively 0·60, 0·70, and 0·53 mm.

From an examination of Figure 4 it will be seen that Planorbis does not oxidise its hæmoglobin immediately on exposure to air. Oxidation starts after 10 minutes and is complete within 20 minutes.

5. Oxygen Debt Experiments.

This investigation was undertaken to find if Planorbis went into oxygen debt when the period of anaerobiosis exceeded that provided for by its reserve of oxygen. The evidence so far obtained indicated that the reserve was sufficient for approximately one hour.

The presence of an oxygen debt following an anaerobic period would be marked by an increase in the oxygen consumption equivalent to the oxygen used by the animal at rest during the same period in air.

The method of measuring the oxygen debt in these experiments was as follows: one snail was weighed and placed in the Barcroft apparatus with

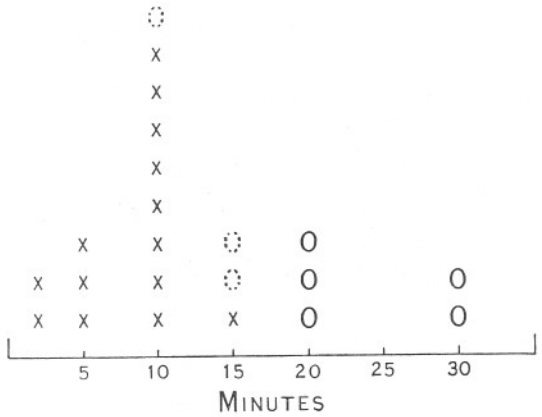

Fig. 4.—Diagram illustrating the time taken for oxidation of the hæmoglobin in separate animals (Planorbis). O, represents the presence of oxyhæmoglobin; ◌, the presence of partially oxidised hæmoglobin; X, the presence of reduced hæmoglobin.

one cubic centimetre of water and some caustic soda to absorb carbon dioxide. The apparatus was kept shaking in a water-bath, at 16° C. Observations were made on the animal's oxygen intake over a period of two to three hours, then by means of pressure tubing connecting the apparatus with the nitrogen supply, suction pump and pressure gauge the apparatus was emptied of air and refilled several times with nitrogen, the snail being left in this anaerobic atmosphere for whatever length of time was required. At the completion of the anaerobic period the apparatus was refilled with air and the oxygen intake again noted for several hours. Commercial nitrogen, before being introduced into the apparatus, was filtered through alkaline hydrosulphite and washed in several alkaline solutions of pyrogallol. Although these precautions were taken to purify the gas a trace of oxygen, amounting to a partial pressure of about 0·5 mm.,

was always present. This did not, however, appear to upset the experiments.

It is important to remember when interpreting the results, that the observed increase in respiration will be due in part to the oxidation of the hæmoglobin and that this amount must, therefore, be subtracted before the true value of the oxygen debt can be ascertained.

Since the average blood volume was estimated at 0·58 c.c. per gm. of snail; the oxygen capacity of the hæmoglobin at 0·013 c.c. per c.c. of

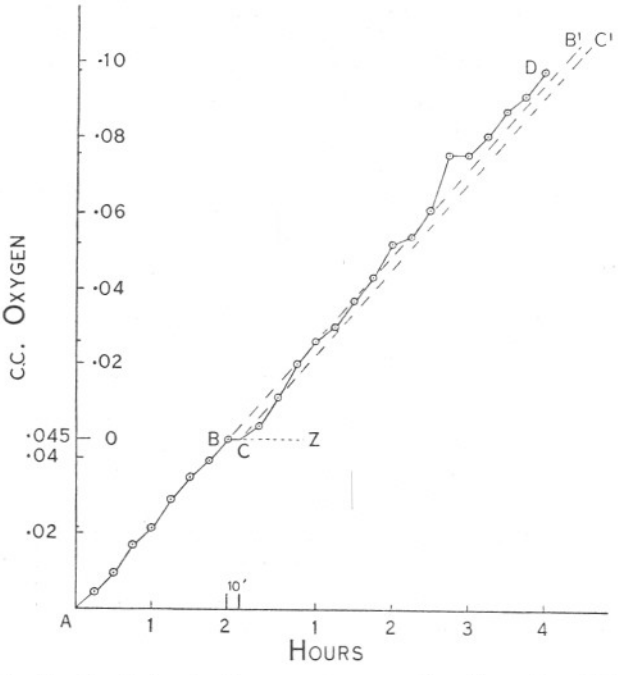

Fig. 5.—Graph showing the oxygen consumption of a snail weighing 0·48 gm. The period of experimental anaerobiosis was 1 hour; the period of true anaerobiosis, 10 minutes; temperature 16·5° C.

blood, it follows that the combined oxygen of the hæmoglobin, 0·0075 c.c. per gm. of snail, will be the amount needed for the oxidation of the hæmoglobin. It was also necessary to calculate the time the combined oxygen would last the animal, this being done for each on the basis of the normal oxygen intake during the experiment. This amount, plus 40 minutes for the supply of oxygen in the lung, was then deducted from the period of experimental anaerobiosis. Thus, if a snail were subjected to two hours oxygen deficiency and the hæmoglobin estimated to have enough oxygen for 20 minutes, the period of true anaerobiosis would be one hour; and from the observed oxygen intake following anaerobiosis was subtracted the

amount needed for oxidation of the hæmoglobin. These corrections were applied to all experiments.

Figures 5, 6, 7, and 8 illustrate typical experiments. The line AB shows the normal oxygen intake before anaerobiosis ; BZ the period of experimental anaerobiosis ; BC the period of true anaerobiosis estimated by deducting from BZ the length of time for which the oxygen in the lung

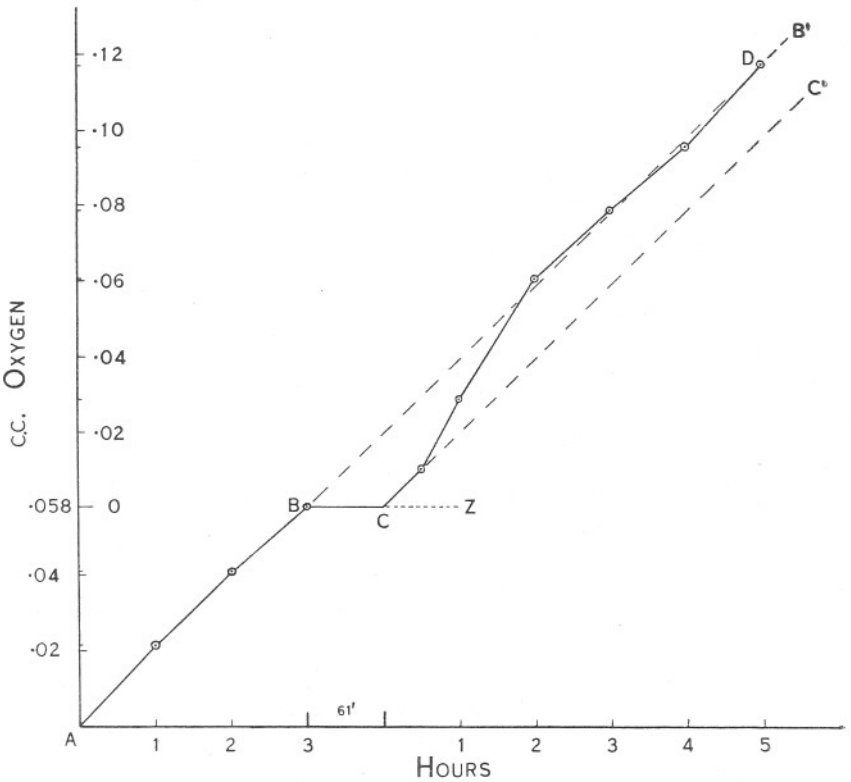

FIG. 6.—Graph showing the oxygen consumption of a snail weighing 0·82 gm. The period of experimental anaerobiosis was 2 hours ; the period of true anaerobiosis, 61 minutes ; temperature 15° C.

and that combined in the blood would last the animal. The broken line BB′ represents the amount of oxygen it is assumed the animal would have used had it not been subjected to anaerobiosis ; the broken line CC′ is that along which post-anaerobic respiration would have proceeded had it been equal to the normal resting value. The line CD shows the oxygen consumption after anaerobiosis less the estimated amount needed for oxidation of the hæmoglobin.

It will be seen from Figures 5, 6, and 7 that when the anaerobic period

has ended, the animal takes up more oxygen than it normally requires, the excess being equal to the amount it would have used during the time it was in nitrogen. Figure 8 illustrates an experiment in which the period of anaerobiosis did not extend beyond the time for which the animal was provided with oxygen by its lung and there was, therefore, no debt.

The results show that for short anaerobic periods up to approximately one hour, the extent to which anaerobiosis was carried, Planorbis went

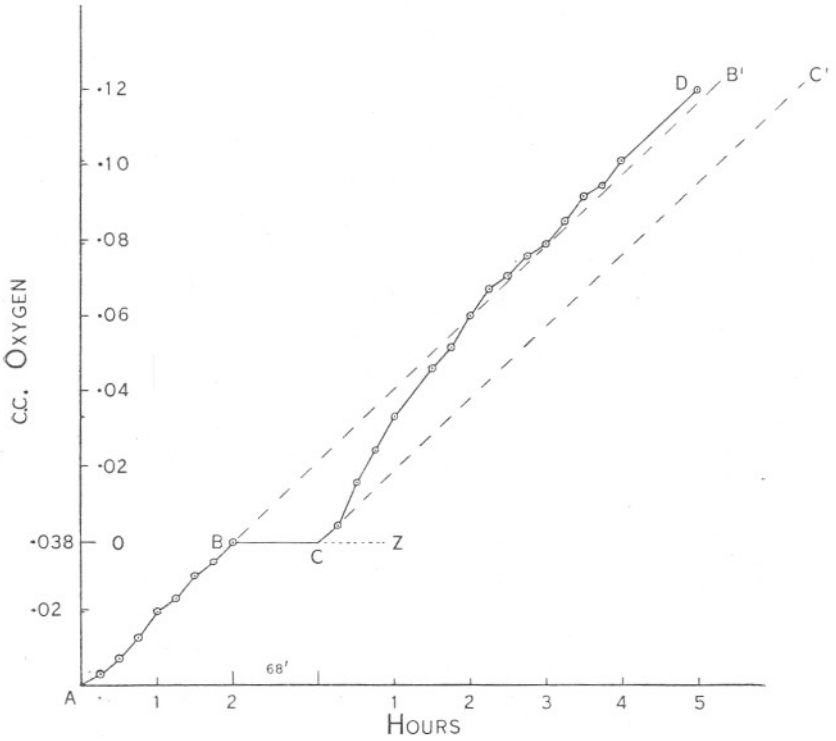

Fig. 7.—Graph showing the oxygen consumption of a snail weighing 0·50 gm. The period of experimental anaerobiosis was 2 hours; the period of true anaerobiosis, 68 minutes; temperature 16° C.

into debt for oxygen. The magnitude of the debt was proportional, within the limits of experimental error, to the time of anaerobiosis; the period of recovery was longer.

6. Discussion.

Planorbis inhabits rivers, canals, ponds and marshes, the water of which may be deficient in oxygen during periods of drought and stagnation. It has been shown by Leitch (1916) that normally enough oxygen is supplied to the snail by diffusion through the surfaces exposed to the

water. When the oxygen pressure falls below that necessary for diffusion the hæmoglobin is reduced and, to facilitate the oxidation of the blood, the animal renews the air in its lung by rising frequently to the surface. The present investigation shows that the combined oxygen is sufficient

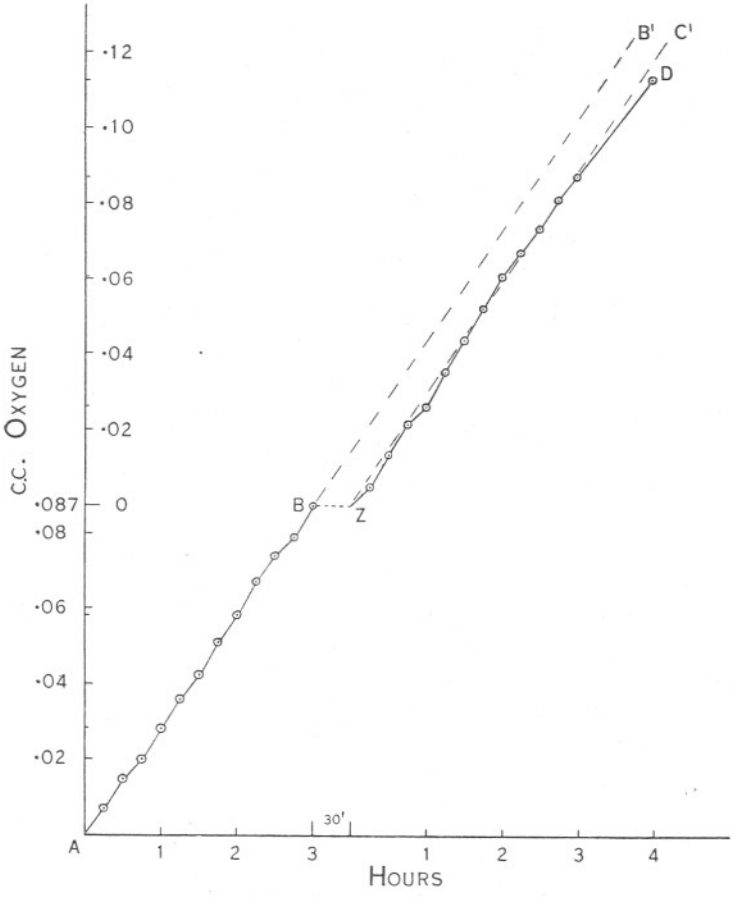

Fig. 8.—Graph showing the oxygen consumption of a snail weighing 0·50 gm. The period of experimental anaerobiosis was 30 minutes. There was no period of true anaerobiosis. Temperature 16° C.

for approximately 25 minutes. Leitch's evidence that the combined oxygen is the last to be used by the animal is further substantiated.

Barcroft (1928) has shown that the main dissociation of Planorbis blood occurs between oxygen pressures of 1 to 10 mm. of Hg.

It is concluded that the hæmoglobin functions primarily as a transporter of oxygen at low pressures. The combined oxygen may be considered as a reserve only in the sense that it supplements the oxygen held

in the lung, with the result that the animal is for a time independent of reduced oxygen pressures in the water. The significance of the hæmoglobin is, therefore, that it furnishes the means whereby oxygen is secured and transported to the tissues at times of lowered oxygen pressure.

The importance of the lung should not be overlooked as, in addition to facilitating the oxidation of the blood, it provides a mechanism by which the animal can obtain oxygen as long as it has access to air.

The investigation establishes the ability of Planorbis to put up an oxygen debt. Planorbis appears, therefore, to be especially well adapted for survival in an environment at times deficient in oxygen.

B. *ARENICOLA MARINA.*

1. Normal Rate of Respiration.

The experimental procedure for the estimation of the oxygen consumption of Arenicola differed only slightly in detail from that already described for Planorbis. Measurements were carried out in Haldane's blood gas apparatus which was kept shaking in a water-bath, the temperature of which ranged between 10° and 12° C., the variation of the temperature for each experiment not exceeding 0·5° C. Carbon dioxide was absorbed by small strips of filter paper moistened with 10% sodium hydroxide solution. The measurements were made on worms varying in weight from 2 to 9 gm., one worm being used at a time and readings taken over a period of six hours. Except for rhythmic contractions of the body wall the worm remained quite still.

The results of six determinations are given in Table VI and show that the average oxygen consumption is of the order of 0·031 c.c. per gm. per hour. The large animals appear to use less oxygen than the small, but yet adult, animals. It seems, therefore, that in the case of Arenicola there may be a relationship between body weight and oxygen intake.

TABLE VI.

No. of Animal.	Weight in gms.	Oxygen Consumption in c.c. per hour. 1	2	3	4	5	6	Average, c.c. per gm. per hr.
1.	2·4	0·080	0·070	0·085	0·100	0·097	0·098	0·037
2.	3·6	0·170	0·150	0·150	0·152	0·118	0·132	0·040
3.	4·3	0·130	0·136	0·140	0·100	0·114	0·115	0·028
4.	5·2	0·125	0·100	0·120	0·090	0·115	0·090	0·020
5.	7·1	0·260	0·240	0·206	0·250	0·220	0·220	0·034
6.	9·5	0·315	0·273	0·252	0·265	0·255	0·245	0·028

In Figure 9 the general character of the results is exemplified by the curve which represents the oxygen used per hour at 11·6° C, by a worm weighing 5·2 gm.

2. Blood Volume.

The blood volume of Arenicola was determined by the same colorimetric method as that outlined for Planorbis. The manner of bleeding the worm was as follows : the worm was opened along the mid-dorsal line to expose the body-cavity : the cœlomic fluid allowed to drain out and the cavity dried as thoroughly as possible : the heart and blood vessels were punctured and the escaping blood removed from the body-cavity with a pipette. In this way it was possible to obtain about 0·5 c.c., the rest of

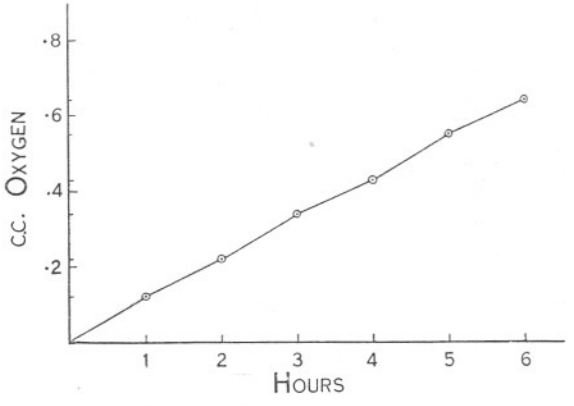

Fig. 9.

the blood being extracted by suction through a Buchner filter after the chopped-up worm had been left standing for some time under water.

In Table VII are given the results from which the average blood volume was estimated to be 0·382 c.c. per gm.

TABLE VII.

No.	Weight of Worm gm.	Volume of Blood c.c.	Volume of Blood per gm. of Worm.
1.	6·65	2·57	0·386
2.	6·87	3·43	0·500
3.	7·49	2·33	0·312
4.	7·70	2·56	0·333

It cannot be claimed that the colorimetric method is absolutely accurate and these results should be considered to be only roughly approximate to the true value. The presence of the black pigment from the epidermis rendered accurate colour comparison difficult.

3. Oxygen Capacity of the Blood.

Estimations of the total oxygen content of Arenicola blood were carried out in the Barcroft and in the Van Slyke apparatus, the procedure being identical with that already described for the experiments on Planorbis blood.

The results given in Table VIII show the oxygen capacity of the hæmoglobin to be 0·087 c.c. per c.c. of blood, the total oxygen content 0·097 c.c. per c.c. of blood.

TABLE VIII.

Estimations made on 1 c.c. of Blood at N.T.P.

Combined Oxygen by Barcroft's Apparatus c.c.	Total Oxygen Capacity of Blood by Van Slyke's Apparatus c.c.
0·097	0·098
0·085	0·097
0·084	0·097
0·085	0·096
—	0·099
—	0·096
Mean 0·087	0·097

The length of time that the total oxygen content of the blood will last the animal may be calculated as follows :—

Blood volume per gm. of worm	0·382 c.c.
Oxygen content per c.c. of blood	0·097 c.c.
Oxygen capacity of blood per gm. of worm	0·037 c.c.
Oxygen consumption per gm. per hour	0·031 c.c.

Hence, oxygen content of blood will last the animal 71 minutes.

4. Spectroscopic Examination of the Hæmoglobin.

(*a*) *Under Anaerobiosis.*

Arenicola blood was examined for the same purpose and by the same method as described for Planorbis. The worms were dried lightly on filter paper before being placed in the nitrogen-box. The process of obtaining the blood was as before, the worms being opened longitudinally, the blood vessels cut and a drop of blood transferred from the body-cavity to a glass slide and covered with a cover slip. The results are expressed in Figure 10.

The first sign of reduction occurred after 20 minutes, while after half an hour the hæmoglobin, although not completely reduced, was as nearly reduced as it was ever found. The analysis of the nitrogen atmosphere in the box showed a partial pressure of oxygen of about 0·5 mm., which

may perhaps explain the failure of the hæmoglobin to appear completely reduced. The dissociation curve of Arenicola hæmoglobin, as given by Barcroft and Barcroft (1924), showed that at this pressure the oxygen saturation of the blood is about 8%.

Since the hæmoglobin was never found in a state of complete reduction

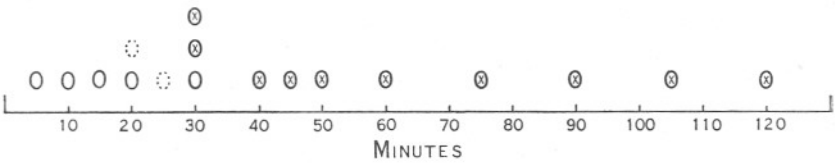

FIG. 10.—Diagram representing the time taken for the reduction of oxyhæmoglobin in Arenicola. Separate animals were examined at frequent intervals. O, indicates presence of oxyhæmoglobin; ⊙, presence of partially reduced hæmoglobin; ⊗, presence of almost completely reduced hæmoglobin.

it is impossible to arrive at any further conclusion concerning the time the oxygen supplied by the blood will last the animal. This investigation shows, however, that the supply is sufficient for at least 30 minutes.

(*b*) *Under Aerobiosis following Anaerobiosis.*

Figure 11 shows the results obtained for Arenicola, the methods being the same as those used for Planorbis and described above.

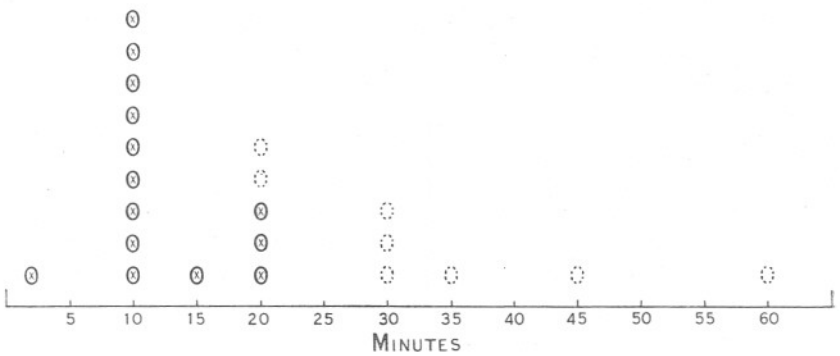

FIG. 11.—Diagram representing the time taken for the oxidation of the hæmoglobin in Arenicola. Separate animals examined at frequent intervals. ⊙, indicates that some oxidation has occurred though it is not complete; ⊗, presence of almost completely reduced hæmoglobin.

It is interesting to note that the hæmoglobin was still in the reduced state after 10 minutes exposure to air, while after 20 minutes, although some oxidation had occurred, well defined bands of oxyhæmoglobin were never found. The explanation for this may be that the temperature at which the experiments were conducted, 20° to 22° C., was higher than that of the

animal's normal environment at that time of the year, which was approximately 15°C. This, coupled with a fairly long exposure to almost complete anaerobiosis, injured the animals to such an extent that recovery did not take place during the time of the experiment.

5. Oxygen Debt Experiments.

Two series of experiments on the ability of Arenicola to put up an oxygen debt were made in the Barcroft apparatus. In the first, the weighed worm was placed in the respiration flask of the apparatus with 1 c.c. of sea-water, and a strip of filter paper moistened with 10% solution of NaOH, to absorb carbon dioxide, was placed in the reservoir, the anaerobic atmosphere being produced as before with nitrogen.

In the second series, hydrogen, washed through sodium hydrosulphite, pyrogallol and silver nitrate solutions, replaced nitrogen. No water was added, the flasks being simply rinsed with sea-water and used moist. Carbon dioxide was absorbed by a small rectangle of filter paper wet with 10% solution of KOH.

The measurement of the oxygen debt was made by observing the normal oxygen intake immediately before and after the period of oxygen lack, anaerobic conditions within the apparatus being attained as before. Analysis of the gas, whether nitrogen or hydrogen, revealed a partial pressure of oxygen of about 0·5 mm.

Observations made during the period of anaerobiosis showed that the volume of gas in the respiration flask gradually diminished, constant volume being attained after about 30 minutes, from which it was concluded that the worm used this small amount of oxygen and was not, therefore, under strictly anaerobic conditions until no more oxygen was available. In a few experiments constant volume was never attained and these were discarded. It was noted that the worm was capable of movement at the completion of the anaerobic period.

To estimate the period of true anaerobiosis allowance was made for the time taken by the worm to use the oxygen impurity in the apparatus and the combined oxygen in its blood. A mean value for the combined oxygen was obtained as follows :—

Volume of blood per gm. of worm	0·382 c.c.
Amount of oxygen combined with Hb per c.c. of blood	0·087 c.c.
Hence, amount of combined oxygen per gm. of worm	0·033 c.c.

The length of time that the combined oxygen will last is dependent on the animal's normal oxygen consumption.

To represent the true value of the debt a deduction was made from the increased oxygen intake following anaerobiosis corresponding to the amount needed by the animal for oxidation of its hæmoglobin. These

corrections were, at the best, only approximate, as they did not allow for individual variation in blood volume and concentration of hæmoglobin in the blood, nor for the fact that before constant volume was attained in the apparatus the worm must have been subjected to partial anaerobiosis.

Some typical results of the oxygen debt experiments are illustrated

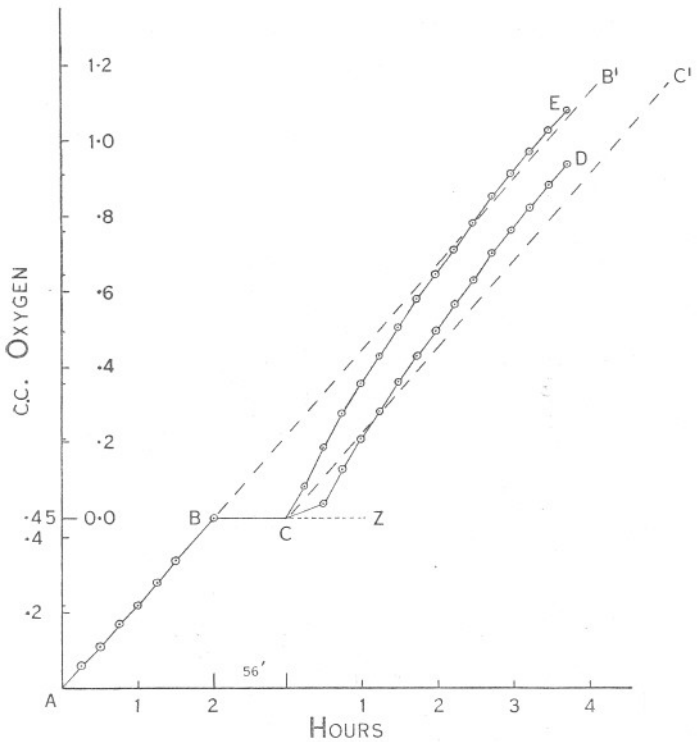

FIG. 12.—Graph showing the oxygen consumption of a worm weighing 4·5 gm. The period of experimental anaerobiosis in hydrogen was 2 hours; the period of true anaerobiosis, 56 minutes; temperature 13° C.

by Figures 12, 13, 14, and 15 in which AB represents the normal oxygen intake measured before anaerobiosis; BZ the period of experimental anaerobiosis; BC the period of true anaerobiosis estimated by deducting from BZ the length of time needed for constant volume to be attained within the apparatus and that for which the combined oxygen of the blood was calculated to last the animal. The broken line BB′ represents the oxygen it is assumed the animal would have used had it not been deprived of air; the broken line CC′ the oxygen intake equal to the normal resting value. The line CD shows the oxygen intake following anaerobiosis, the

correction for the oxidation of hæmoglobin having been applied ; while CE is the total oxygen intake following anaerobiosis as measured during the experiment.

Figures 12 and 13 illustrate experiments in which a partial debt was found. It will be noticed that the excess oxygen intake was equal to about half what the animal would have used during the same time in air. Figure 14 is typical of the results of the majority of experiments in which there appeared to be no oxygen debt. Figure 15 illustrates an experiment in which the period of experimental anaerobiosis was 30 minutes, the

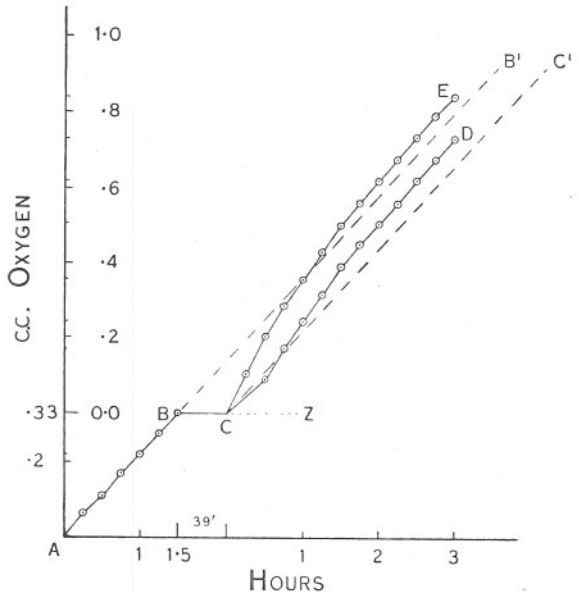

Fig. 13.—Graph showing the oxygen consumption of a worm weighing 3·4 gm. The period of experimental anaerobiosis in hydrogen was 1 hour 36 minutes; the period of true anaerobiosis, 39 minutes; temperature 14° C.

oxygen impurity in the apparatus enough for 3 minutes and the combined oxygen in the blood sufficient for 40 minutes. The worm was, therefore, not under anaerobic conditions as far as its blood was concerned, and thus there should be no debt, as was found to be the case when the amount needed for the oxidation of the hæmoglobin was subtracted from the observed oxygen intake.

The investigation on the ability of Arenicola to go into oxygen debt has given, on the whole, inconclusive results. The first series of experiments, in which nitrogen was used, indicate that the oxygen consumption after anaerobiosis was below normal. The second series of ten experiments

with hydrogen showed two instances of a partial oxygen debt. If, however, no deduction for the oxidation of the hæmoglobin is made, two out of the six experiments in which nitrogen was used show an oxygen debt, the remaining four a partial debt. Five of the experiments with hydrogen give a debt and four others only a partial oxygen debt.

It seems reasonable to conclude that Arenicola will oxidise its hæmoglobin in the course of one or two hours. Although the correction allowed for this may be too great, as is indicated by the experiments in which the

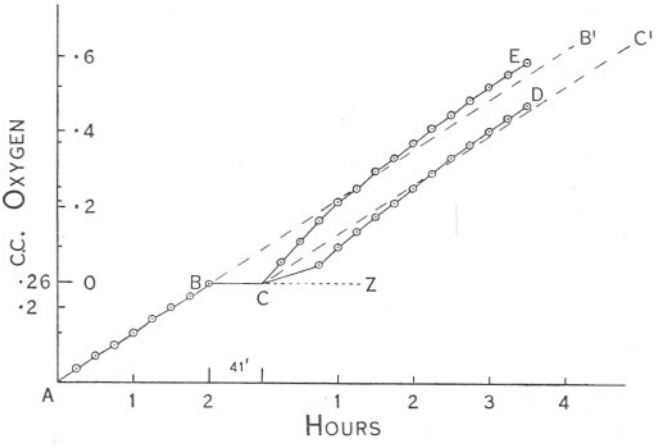

FIG. 14.—Graph showing the oxygen consumption of a worm weighing 3·6 gm. The period of experimental anaerobiosis in hydrogen was 2 hours; the period of true anærobiosis, 41 minutes; temperature 13° C.

corrected value for the oxygen intake falls below normal, the failure of the majority to show an oxygen debt cannot be entirely due to the error introduced by this correction.

6. The Habitat and Habits of Arenicola.

The worms used for this research were collected from a sandy beach forming part of Batten Bay, on the east side of Plymouth Sound. The area inhabited by Arenicola is uncovered between high and low tides for approximately three hours. The sand is black, with the exception of the surface layer, which is brown to a depth of one-quarter to one-half an inch. It is well known that black sand indicates the presence of sulphides from decomposing organic matter, while the brown colouration is the result of the oxidation of sulphides by the atmosphere. Arenicola burrows to a depth of one to two feet below the surface. The burrow, which is U-shaped and open at each end, is constructed by the animal pushing and eating its way through the sand. The entrance is not sealed at low tide by the castings. These are, in general, coiled in a heap around the opening in

such a manner that the latter is clearly visible. It was observed that whereas the surrounding sand is black that lining the burrows is brown, similar to the surface sand. The brown layer is approximately 1 mm. in thickness and extends throughout the entire length of the burrow.

The oxygen content of the interstitial water was determined as follows. Samples of the water from one foot below the surface were taken from a marked area on three occasions, each sample consisting of four lots of water collected at intervals of approximately one hour, so that the first was obtained just as the tide uncovered the area and the fourth just as the tide again reached the area. The boring apparatus used to obtain the

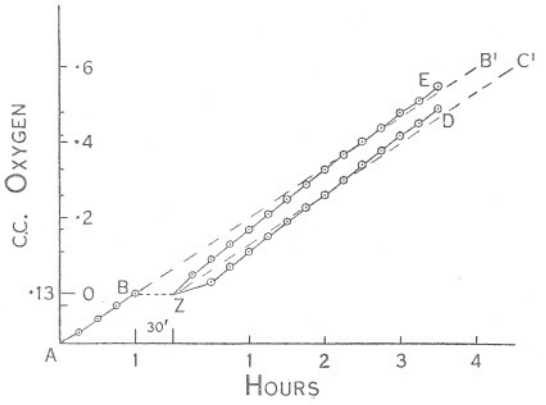

FIG. 15.—Graph showing the oxygen consumption of a worm weighing 2·8 gm. The period of experimental anaerobiosis in nitrogen was 30 minutes. There was no period of true anaerobiosis as the worm had a supply of oxygen sufficient for 43 minutes. Temperature 11° C.

samples consisted of a hollow iron tube, two inches in diameter and two and a half feet in length, closed at the bottom and terminating in a spiral spike. Near the bottom was a double row of holes, 5 mm. in diameter, blocked on the inside by means of a plunger which could be withdrawn as desired. The borer was forced into the sand to the required depth and the plunger then removed so that the water could drain in through the holes. The water was obtained by sucking it up into a large separating funnel the stem of which reached to the bottom of the borer. Three successive samples of water were thus collected and run into bottles of approximately 250 c.c. capacity. Precautions were taken to keep the water as free as possible from contact with air by sampling under liquid paraffin.

The estimation of oxygen was made in the laboratory by Winkler's method. Time was first allowed for the sediment invariably drawn up

with the water to settle. This was necessary as the sediment contained sulphides which on the addition of acid liberated hydrogen sulphide, the latter combining with the iodine from Winkler's reagents. The bottles were then opened and water free from sediment, drawn off from the upper layer, was transferred under oil to smaller bottles of 60 c.c. capacity. The estimations of dissolved oxygen were then carried out.

The remaining water in the large bottles was tested for hydrogen sulphide by adding acid to samples of water free from sediment and to other samples well mixed with sediment, and testing these with lead acetate paper. It was found that the water which had been shaken up with sediment generally gave a positive test for hydrogen sulphide, whereas that lacking sediment invariably gave a negative test. It was, therefore, concluded that the method of allowing sediment to settle before estimating the oxygen was sufficient to prevent the interference of hydrogen sulphide with Winkler's reagents.

Control experiments were devised to find the margin of error introduced into the oxygen estimations by the unavoidable handling of the water in the field. For this purpose sea-water was boiled to expel air and samples siphoned into several of the 60 c.c. bottles. The rest of the boiled water was then sucked up into the separating funnel, run into 250 c.c. bottles, and samples transferred from them to other 60 c.c. bottles under oil. The technique was as far as possible an exact reproduction of that employed in the sampling of water from the field. The oxygen concentration was determined by Winkler's method. The difference between that found in the siphoned samples and that in which the water was transferred by the separating funnel represents the error introduced by the handling of the water. The average error introduced by this method of sampling the water was found to be 0·44 c.c. of oxygen per litre. Table IX gives the oxygen content in c.c. per litre of the interstitial water.

TABLE IX.

Date of Sampling.	Oxygen content of water from sand in c.c. per litre at Exposure.	1 hour.	2 hours.	3 hours.
Oct. 24.	0·107	0·058	0·142	0·145
	0·066	0·151	0·137	0·034
	0·114	0·125	0·165	0·028
Nov. 7.	0·137	0·021	0·136	0·130
	0·126	0·055	0·124	0·197
	0·114	0·073	0·241	—
Nov. 10.	0·212	0·220	0·130	0·266
	0·199	0·135	0·151	0·125
	0·218	0·117	0·206	0·143

The highest value of oxygen found was 0·27 c.c. per litre, and as the experimental error was 0·44 c.c. per litre it is clear that there is practically no oxygen in the interstitial water at any time. The variation in the values of oxygen present in samples collected at a given time may, perhaps, be explained by the amounts of sediment in the bottles being different, and by the difference in the length of time the water was exposed to the sediment.

The data so far presented show that the worms burrow in black sand, the interstitial water of which at a depth of 1 foot lacks oxygen. The burrows, however, are lined with brown sand, indicating that oxidation has occurred.

A few observations have been made in the laboratory on the burrowing habits of Arenicola. It was noticed that in the process of burrowing the worms acted as small suction pumps, that is, by everting and inverting the proboscis water was drawn towards the anterior end from all sides. The worms begin their burrows when covered with water and it is clear that as they move into the sand water will flow in behind them, thus oxidising the lining of the burrow which consists of particles of sand held together with mucus and forming a layer about 1 mm. in thickness. It was noted in one case where a worm had died in its burrow and lost blood that the red colouration did not diffuse into the surrounding water. This indicates that the water in the burrows cannot be considered as part of the interstitial water.

It is presumed that when the tide is high the worms have no difficulty in obtaining oxygen, as they can either maintain a current through the burrow or come into direct contact with fresh sea-water by moving to the surface.

During the period of intertidal exposure they are most generally found at a considerable depth below the surface. It is probable that during this period, which lasts for approximately three hours, conditions approaching anaerobiosis will occur. The water in a completed burrow was observed in the laboratory to move and to change its direction of motion suddenly, as the worm alternately protruded and withdrew its proboscis. Fine particles of sediment were observed in motion within the burrow. It is, therefore, assumed that the movement of the worm within its burrow will keep the water in a state of constant motion, thus bringing it all into contact with the air at the surface. Since the openings are not more than 0·5 cm. in diameter the surface of water exposed to the air is obviously restricted and thus only a limited amount of oxygen can be dissolved in a given time. This amount will, however, be used by the worm as the dissociation of its blood occurs mainly at very low oxygen pressures. It is thought that the oxygen thus acquired, in conjunction with that already combined with the hæmoglobin, will be sufficient to satisfy the animal's requirements during low tide.

7. Discussion.

Barcroft and Barcroft (1924) have shown that the dissociation of Arenicola blood takes place mainly at oxygen pressures of between 1 and 3 mm. of Hg. It is evident that in well aerated water respiration is effected by the diffusion of oxygen through the gill filaments into the blood stream, the transport of oxygen by the hæmoglobin taking place only at reduced pressures.

The great affinity of the hæmoglobin for oxygen and the consequent low pressure at which it dissociates adapt Arenicola particularly well to its environment.

It is concluded that the primary function of the hæmoglobin is that of transporting oxygen during the period of lowered oxygen pressures to which the worms are probably subjected at low tide. The theoretically calculated amount of combined oxygen is enough for an hour and, while it is not suggested that the hæmoglobin functions chiefly as a storer of oxygen, the reserve it holds will be of great service to the animal.

The experiments on the ability of Arenicola to go into oxygen debt gave on the whole unsatisfactory results. In a few instances the oxygen intake following anaerobiosis was in excess of the normal. This would seem to indicate that Arenicola is able to go into debt for oxygen, but that its ability to do so is limited. The limiting factors may perhaps be a low buffering power on the part of the tissues and blood. The concentration of lactic acid was not estimated. In view of the results obtained this will be necessary before it is possible to conclude to what extent the mechanism of the oxygen debt is of use to the animal.

SUMMARY.

A study of the respiration and of the function of hæmoglobin in *Planorbis corneus* and *Arenicola marina* has been undertaken.

The oxygen consumption of Planorbis is of the order of 0·026 c.c. per gm. per hour, measured at 15° C.

The blood volume is approximately 0·581 c.c. per gm.

The combined oxygen of the blood is 0·013 c.c. per c.c. of blood, the total oxygen being 0·014 c.c. per c.c. The total oxygen capacity of the blood per gm. of snail is estimated to be 0·0081 c.c.

The oxygen supplied by the blood is calculated to last 18 minutes and is found by experiment to last 25 minutes.

The snail does not immediately oxidise its hæmoglobin after subjection to anaerobiosis. Oxidation begins after 10 minutes and is complete within 20 minutes.

Planorbis goes into debt for oxygen when subjected to short anaerobic

periods. The debt is proportional to the time of anaerobiosis, but the recovery period is longer.

This animal appears to be well adapted for survival in a habitat which at times may be deficient in oxygen.

The function assigned to the hæmoglobin is that of transporting oxygen.

The oxygen consumption of Arenicola is of the order of 0·031 c.c. per gm. per hour, measured at between 10° and 12° C.

The blood volume is approximately 0·382 c.c. per gm.

The combined oxygen of the blood is 0·087 c.c. per c.c. of blood, the total oxygen content being 0·097 c.c. per c.c. The total oxygen capacity of the blood is estimated to be 0·037 c.c. per gm. of worm.

The oxygen supply of Arenicola blood is calculated to last 71 minutes and is found by experiment to last at least 30 minutes.

Arenicola when transferred from anaerobic to aerobic conditions partially oxidises its hæmoglobin after 20 minutes. Instances of complete oxidation were never found.

The results of the oxygen debt experiments are not conclusive. A few instances of a partial debt were found. It is thought that the ability of the worm to go into oxygen debt is limited.

The sand at Plymouth, in which the Arenicola burrow, is black, while that lining the burrows is brown, similar to the surface sand. The burrows open to the surface at each end and the openings are not blocked by the castings. The amount of oxygen in the interstitial water is found to be negligible at all times. The water in the burrows is not considered to form part of the interstitial water. It is thought that the movements of the worm within the burrow keep the water in motion so that oxygen is being continually renewed at the surface.

The dissociation of Arenicola hæmoglobin occurring at low oxygen pressures of between 1 and 3 mm. of Hg. seems especially adapted to the needs of the animals. The significance of the hæmoglobin is that it functions as a carrier of oxygen during the period of lowered oxygen pressures to which the animals are liable to be subjected during intertidal exposure.

Acknowledgments.

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